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Publication Date

2020-03-01

DOI

10.1016/j.geoderma.2019.114111

Peer reviewed

Title: Exotic herbaceous species interact with severe drought to alter soil N cycling in a semi-arid shrubland

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Keywords: Climate change, Exotic species invasion, Coastal sage scrub; Soil nitrogen

Abstract

Mediterranean-type ecosystems are increasingly threatened by climate change and exotic annual species, jeopardizing the native communities and their global biodiversity. In these systems, soil nitrogen (N) limits net primary production, and its availability can be influenced by both of these stressors. To understand the interactive effects of droughts and exotic herbaceous species on soil N, we monitored the temporal variability of soil inorganic N, net N mineralization, net nitrification, and N leaching under native- and exotic-dominated stands exposed to rainfall manipulation plots in a Mediterranean-type shrub-dominated community. Increasing drought severity resulted in the accumulation of soil NH_4^+ and NO_3^- , with a more pronounced increase in exotic-dominated plots. Increased net N mineralization and net nitrification and reduced leaching losses were observed as mechanisms of inorganic N accumulation. In comparison to soils under native plants, soils under exotic plants had enhanced leaching losses upon soil rewetting. We propose that distinct traits of exotic annual herbaceous species associated with higher N inputs, faster turnover, and reduced temporal uptake determine the changes in N cycling in response to droughts. Severe droughts and exotic plants may produce a larger, more vulnerable pool of N that is prone to losses while providing a competitive advantage to promote exotic growth in these N-limited ecosystems.

Introduction

Nitrogen (N) cycling is being altered globally more than any other essential nutrient (Fields, 2004). Agriculture and fossil fuel combustion are increasing reactive N in the

atmosphere (Galloway et al., 2008), droughts are increasing the ecosystems' N losses (Homyak et al., 2017), and invasive plants are altering ecosystems' N pools and fluxes (Ehrenfeld, 2003; Liao et al., 2008). These N alterations represent a major challenge particularly in ecosystems where N limits net primary production (Delgado-Baquerizo et al., 2014), and its availability is being threatened by multiple co-occurring global change drivers (Chapin et al., 2000; Sala et al., 2000). For example, semi-arid shrublands are currently experiencing invasions by exotic herbaceous species (Minnich and Dezzani, 1998; Yu et al., 2016), more intense and prolonged droughts (Spinoni et al., 2014; Swain et al., 2018), and elevated N deposition (Valliere et al., 2017), constituting multiple stressors that may have large impacts on N cycling.

In semi-arid ecosystems, N's limited accessibility is controlled by the temporal dynamics of water availability (Reichmann et al., 2013; Shen et al., 2008). During wet periods, N becomes accessible for microbial and plant assimilation and transformations, which contrasts to natural periods of drought when decreases in diffusion and biological uptake result in the accumulation of immobile N replenished from dead biomass and dry deposition (Cregger et al., 2014). The synchronized immobilization by microbes and plants during wet periods coupled with limited diffusivity during dry periods promotes a closed N cycle (recycling) in these systems (Homyak et al., 2016; Joergensen and Wichern, 2018). However, an asynchrony/decoupling between soil N availability and plant and microbial processes creates N leaks, particularly when dry soils rewet outside of the plant growing season (Homyak et al., 2016). Drought-tolerant microbial transformations and a lack of plant demand during ephemeral periods with mobile N availability favors N losses via leaching and gaseous pathways (Dijkstra et al., 2012). However, losses due to

64 this open N cycling have been reported to be low or limited to intermittent rain pulses in semi-
65 arid ecosystems (Evans and Burke, 2012; Jongen et al., 2013).

66
67 More intense and prolonged droughts due to climate change are expected to promote
68 open N cycling in semi-arid shrublands via sustained N processing during periods of low plant
69 uptake (Evans and Burke, 2012; Homyak et al., 2016). Invasions by exotic herbaceous species
70 have also been reported to increase soil N availability and cycling rates (Blank, 2008; Hawkes et
71 al., 2005; Wolkovich et al., 2010) by possessing traits associated with faster N turnover (Castro-
72 Díez et al., 2014). In semi-arid ecosystems this excess of N availability has been associated with
73 detrimental environmental effects, such as increasing emissions of nitrogenous greenhouse gases
74 (Homyak et al., 2016), eutrophication, fire frequency (Fenn et al., 2003), and competitive
75 advantages for exotic plant species (Laungani and Knops, 2009; Liu et al., 2018). While soil N
76 cycling alterations in response to droughts and plant invasions in semi-arid shrublands have been
77 evaluated, a mechanistic understanding of the interactive effects of these co-occurring global
78 change drivers is not only limiting our ability to model ecosystem responses to multiple global
79 change drivers but also limits improvements to conservation efforts aimed at maintaining
80 ecosystem services (Alba et al., 2017; Laungani and Knops, 2009; Valliere, 2016). Levels of
81 uncertainty arise from challenges in the evaluation of temporally dynamic abiotic and biotic
82 drivers (Alba et al., 2017) and the intrinsic variability of dryland ecosystems that are
83 characterized by distinct plant community types (Fenn et al., 2003) and hydrologic regimes
84 (D'Odorico and Bhattachan, 2012).

Here we present the results of a four-year rainfall manipulation field study in a coastal sage scrub community (CSS), an understudied semi-arid Mediterranean-type, shrub-dominated ecosystem distributed from coastal central California, USA to northern Baja California, Mexico, that has been invaded by exotic herbaceous species (Minnich and Dezzani, 1998) and experienced multi-year extreme drought conditions (Swain et al., 2018). Established exotic annual grasses and forbs species differ from dominant CSS native perennial semi-deciduous shrubs not only in growth form and phenology but also in distinct drought strategies (Balachowski et al., 2016; Puritty et al., 2019; Wainwright et al., 2012; Wolkovich et al., 2010). While CSS native shrubs are adapted to persist through seasonal droughts by partial senescence of mature foliage that is released late in the dry season, exotic annual species exhibit faster growth and earlier complete senescence before experiencing dehydration (Balachowski et al., 2016). The monitoring of temporal variability of soil inorganic N, net N mineralization, net nitrification, and NO_3^- leaching under native- and exotic-dominated rainfall manipulated plots allowed us to assess interactive mechanisms of soil N alterations. Combining field manipulations and functional trait approaches has become a powerful tool to improve our mechanistic understanding of ecosystem responses and feedbacks to multiple global change drivers (Boyd et al., 2018; Laungani and Knops, 2009). We hypothesized that the earlier senescence of exotic species, and their more easily decomposable litter compared to native species, would enhance soil N availability and result in more open cycling in response to droughts (Liao et al., 2008).

Materials and Methods

This study was conducted from 2013 – 2016 at the Santa Margarita Ecological Reserve (SMER) rainfall manipulation experiment (33.44128 N, 117.1644 W), located at the Riverside-San Diego county line, California, USA. The region has a semi-arid Mediterranean-type climate with cool wet winters and hot dry summers with a compared 30-year average of 358 mm yr⁻¹ of rainfall. The site was a grazed pasture prior to the establishment of the Reserve in 1962. Currently, the area is a heterogeneous matrix of native mature shrubs such as *Artemisia californica* and *Salvia mellifera* interspersed with exotic annual species such as *Centaurea melitensis*, *Hirschfeldia incana*, *Erodium cicutarium* and *Bromus madritensis*. All plots were located on similar soil, classified as Las Posas loam, which are well drained with an argillic horizon under the top soil (<http://websoilsurvey.sc.egov.usda.gov>). Soil analyses at the beginning of the experiment showed no differences in physicochemical properties between plots (Table 1). We are therefore confident that the effects observed in this experiment did not arise from pre-experimental variability among plots. The field experiment consisted of 30 3 m x 3 m rainfall shelters covered with a clear, polycarbonate roof permitting light transmission. Half of the plots were dominated by the native mature shrubs, while the remaining plots were dominated by exotic annual species. To implement the rainfall manipulation, rainfall collected from the rain shelters was re-applied to plots using sprinkler heads at three rainfall levels (in each vegetation type, 5 replicates of 50%, 100%, and 150% of ambient rainfall) after rainfall events. During the sampling period, the region experienced below average annual rainfall with the ambient rainfall plots receiving 157, 125, 139, and 156 mm yr⁻¹ during water years 2013 – 2016, respectively. Due to the lack of rainfall, the 50% and 100% rainfall treatments represented severe drought and drought conditions, respectively, and the 150% treatment corresponded to an average water year.

Water year 2015 received major Pacific storms and summer rains associated with the 2015 – 2016 El Niño event.

N pools

To evaluate temporal variability in soil inorganic N, we sampled soils from all plots during the winter (rainy season: October-February), spring (peak plant biomass: March-April), and summer (dry season: May-September) over the 4-year sampling period, resulting in 12 sampling events. Soil was sampled to a depth of 15 cm using a 5 cm diameter x 15 cm deep slide hammer soil core sampler. Samples were stored in a cooler for transportation and processed within 24 h of collection. All visible stones, roots, and litter were removed with forceps prior to analysis. Dissolved NH_4^+ and NO_3^- were determined in 0.5M potassium sulfate extracts using the indophenol method and by vanadium (III) reduction, respectively, and measured on a spectrophotometer (Miranda et al., 2001).

Net N mineralization, net nitrification and leaching

Net N mineralization and nitrification were assessed using in situ soil core incubations with 4 cm diameter and 10 cm depth PVC tubes (DiStefano and Gholz, 1986). Two cores were inserted into the soil inside the plots of 3 out of the 5 replicates for each plant community type and each precipitation manipulation ($n_{natives}=9$ vs. $n_{exotics}=9$) at the beginning of the winter

seasons. One of the cores was immediately removed for analyses (T_0) and the other core, which was fitted with an ion exchange resin bag at the bottom of the tube, was removed during the summer season (T_F). Dissolved NH_4^+ and NO_3^- were analyzed in the potassium sulfate extracts from soils in the cores and in 2 M KCl extracts from the ion exchange beads in the resin bags. Annual N cycling measurements included: (1) N mineralization, calculated as the difference in DIN ($NH_4^+ + NO_3^-$) concentrations between T_F and T_0 plus the DIN in resin bags, (2) nitrification, calculated as the difference in NO_3^- concentrations between T_F and T_0 in resin bags, and (3) leaching as the amount of DIN in resin bags (T_F). Although resin bags may not retain all NH_4^+ and NO_3^- flowing down the core, the amount of DIN in resin bags provides a reliable estimate of N leaching (Bhagal et al., 1999). To compliment leaching measurements, immediately after the 2015 summer storms, we collected soil samples to a depth of 50 cm and measured dissolved NO_3^- on water extracts using ion-chromatography (Thermo ICS-5000+).

Statistical analyses

Data analysis was performed in R version 3.3.3 (R Core Team, 2017). To determine the effects of rainfall treatment, plant community type, season, and year and their interactions on soil NH_4^+ , NO_3^- , N mineralization, nitrification, and leaching, mixed-effects repeated measures analysis of variance (RM-ANOVA) was employed using the lme function within the 'nlme' package (Pinheiro et al., 2018). Linear model assumptions of normality and homoscedasticity were tested using Shapiro-Wilk and Levene's test and observed visually using Q-Q plots and plotting residuals against fitted values, respectively. When significant effects were found, multiple comparisons between treatments, seasons, and years were performed using the glht

function within the ‘multcomp’ package (Hothorn et al., 2008). P-values less than 0.05 were considered significant.

Results

Nitrogen pools

We detected significant differences in soil NH_4^+ and NO_3^- concentrations between years ($p_{NH_4^+} = 0.0002$ and $p_{NO_3^-} < 0.0001$), seasons ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.01$), rainfall treatments ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.006$), and plant community types ($p_{NH_4^+} = 0.02$ and $p_{NO_3^-} < 0.0001$; Table 1). Mean annual soil NH_4^+ and NO_3^- concentrations increased over the 4-year sampling period under both plant community types, with a more pronounced increase in exotic-dominated plots (Fig. 1a-b). In 2016, annual average soil NH_4^+ was 3.1 and 2.5 times higher in exotic- and native-dominated plots, respectively, when compared to 2013 (Fig. 1a). Annual average soil NO_3^- was 6.0 and 8.4 times higher in native- and exotic-dominated plots, respectively, in 2016 when compared to 2013 (Fig. 1b). Increases in soil NH_4^+ and NO_3^- concentrations were also observed during the winter season (Fig. 2a-b) and in the 50% rainfall treatment under both plant communities (Fig. 3a-b). Soil NH_4^+ in the 50% treatment (severe drought) was 2.0 and 1.3 times higher in exotic- and native-dominated plots, respectively, when compared to the 150% treatment (Fig. 3a). Soil NO_3^- was 2.0 and 1.8 times higher in exotic- and native-dominated plots, respectively, in the 50% treatment when compared to the 150% treatment (Fig. 3b). Soil NH_4^+ concentrations were increased in exotic plots by approximately 43% based

on average values across the entire time of observations (estimated mean \pm SD, native-dominated: $2.4 \pm 3.6 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $3.4 \pm 4.2 \mu\text{g N g}^{-1}$ soil). Soil NO_3^- concentrations were increased in exotic plots by approximately 219% (estimated mean \pm SD, native-dominated: $2.2 \pm 3.2 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $7.0 \pm 8.2 \mu\text{g N g}^{-1}$ soil).

Net N mineralization, net nitrification and leaching

Net N mineralization was influenced by year ($p = 0.03$), and there was a moderately significant interaction between rainfall treatment and plant community type ($p = 0.07$; Table 1). Annual net N mineralization was more variable in exotic-dominated plots (Fig. 1c). In exotic plots, net N mineralization rates increased from 2014 to 2015 and decreased from 2015 to 2016. Highest net N mineralization occurred in 2015 in exotic-dominated plots (Fig. 1c). During 2016, net N mineralization was negative (i.e. net immobilization) in exotic plots (Fig. 1c). Net N mineralization differed between the two community types in response to drought. In exotic-dominated plots net N mineralization increased with increasing drought intensity, while in native plots the opposite pattern was present with the highest levels observed in the 150% treatment (Fig. 3c).

While we did not detect significant effects of rainfall treatment, plant community type, or year on net nitrification (Table 2), similar patterns to net N mineralization rates were observed (Figure 1d & 3d). Highest net nitrification rates occurred in 2015 in exotic-dominated plots, and

negative net nitrification was observed in 2016 (Fig. 1d). The response of net nitrification to drought differed in direction between the two community types, although this was non-significant. In exotic-dominated plots net nitrification increased with drought intensity, and in native plots highest levels of net nitrification were found in the 150% treatment (Fig. 3d).

Leaching (DIN in resin bags) was significantly influenced by the interaction between plant community type and year ($p < 0.0001$). In soils under native plants, leaching was higher during 2015 when compared with 2013, 2014, and 2016 (Fig. 4a). In soils under exotic plants, leaching was higher during 2015 and 2016 when compared with 2013 and 2014 (Fig. 4a). During 2015 and 2016 leaching was 1.9 and 4.1 times higher in exotic-dominated plots when compared to native plots, respectively. After the 2015 summer rains we measured a 1.9, 3.9, 5.0, 6.7, and 16.4 times increase in soil dissolved NO_3^- concentrations in exotic plots when compared to native plots at 0-10, 10-20, 20-30, 30-40, and 40-50 cm soil depths, respectively (Fig. 4b).

Discussion

Soil inorganic N accumulation under drier conditions

Consistent with the generalization that drier conditions facilitate inorganic N accumulation (Evans and Burke, 2012; Homyak et al., 2017, 2016), in this study we found higher levels of soil NH_4^+ and NO_3^- concentrations in response to increasing drought severity due both to rainfall manipulations and over the 4-year period of below average annual rainfall under both

plant community types. We observed several patterns that provide insight into the mechanisms of soil inorganic N accumulation. First, annual rates of net N mineralization and nitrification rates were observed to be resistant to drought severity on average (rainfall treatments, Table 2), and net nitrification did not differ significantly during the 4 consecutive years of below average annual rainfall, suggesting statistically unaltered contributions to the supply of NH_4^+ and NO_3^- concentrations during dry periods. The resistance of microbial processes to low water potentials has been reported particularly in dryland ecosystems due to their natural exposure to seasonal droughts (Canarini et al., 2017; Drigo et al., 2017). However, it is also possible that there were seasonal changes in N cycling rates that were not detected with our annual measures. Increased inorganic N in response to drought may have also been caused by other drought-tolerant microbial N sources, such as asymbiotic N fixation, (Boring et al., 1988), or N deposition, particularly dry deposition (Valliere et al., 2017). In the location of our study, N deposition occurs at levels ranging from 9-11 kg N $ha^{-1}yr^{-1}$ (Fenn et al., 2010). Secondly, we observed a significant increase in leaching during 2015 (wettest year) when compared to previous years under both plant community types. This pattern suggests inorganic N accumulates through reduced leaching losses during drier conditions. Nitrate is highly mobile, and its diffusivity has been previously reported limited under drought conditions (Evans and Burke, 2012). Lastly, differences in soil inorganic N concentrations and leaching between exotic- and native-dominated plots suggest that plant-derived mechanisms influence N accumulation.

Soil inorganic N accumulation as affected by invasion

In comparison to soils under native plants, soils under exotic herbaceous species were observed to have higher soil inorganic N accumulation. We propose that exotic herbaceous species increased soil inorganic N availability by making N cycling more rapid. Litter N release may have been faster from the exotic herbaceous species because of their higher litter quality and their earlier and complete senescence of biomass. This explanation is consistent with the generalization that invasive plants increase N pools by possessing traits associated with faster N turnover (Castro-Díez et al., 2014; Liao et al., 2008). Additionally, invasion by exotic species may accelerate N release to soils via increased litter susceptibility to UV photodegradation compared with native litter (Esch et al., 2019a). Vegetation structure could also play a role, aboveground cover by exotic herbaceous species when compared with native shrubs could enhance the effects of solar radiation on litter decomposition. Plant traits associated with N-fixation have been proposed as another mechanism by which invasive plants enhance N pools (Castro-Díez et al., 2014). While not evaluated in this study, higher asymbiotic N fixation activity may be a mechanism by which the non N-fixing exotic species could have increased soil N inputs (Castro-Díez et al., 2014; Eisele et al., 1989; Mack et al., 2001). An increase in the relative abundance of Cyanobacterial DNA sequences has been found in our exotic-dominated plots (Pérez Castro et al., 2019), which are associated with N fixation (Green et al., 2008).

The interaction of invasion and dry conditions

Contrary to soils under native-dominated plots, we observed a moderate positive relationship between drought severity and N accumulation and net transformations under exotic-dominated plant communities. We proposed that accelerated N cycling by exotic herbaceous

species coupled with drought-induced increases in N accumulation generated a larger inorganic N pool. However, significant decreases in soil NH_4^+ from 2014 to 2015 (the relatively wet year), and increases in N leaching and higher deep soil NO_3^- levels during 2015 in soils under exotic species when compared to soils under native species, suggest that soils under exotic plants did not retain the accumulated N. We speculate that the shorter phenology of the exotic annual species (Esch et al., 2019b) and reduced N uptake (demand) during water inputs increased its vulnerability to leaching losses. This is consistent with the hypothesis that drought-induced soil inorganic N accumulation is more vulnerable to losses upon rewetting (Evans and Burke, 2012).

Conclusions

In this experimental semi-arid shrubland, we found that plant functional traits are key factors in providing mechanistic explanations of soil responses and feedbacks to environmental disturbances. In comparison to soils under native woody plants, we observed that soils under exotic herbaceous plants had enhanced inorganic N accumulation and leaching losses after experiencing severe drought conditions. Our findings indicate that distinct morphological, physiological, and phenological traits of annual herbaceous species, associated with N higher inputs, faster turnover, and reduced temporal uptake, play a major role in N pools and fluxes. Nitrogen alterations under severe droughts and exotic plants likely produce a larger pool of N that is more vulnerable to increased leaching and may provide a competitive advantage to promote exotic growth and invasiveness success in these N-limited ecosystems (Liu et al., 2018; Vourlitis, 2017). While this study suggests N transformation rates as mechanisms of inorganic N accumulation, future efforts that measure gross N production are needed to confirm this speculation and better understand microbially mediated N-cycling processes.

307

308 **Acknowledgments**

309 We thank Rachel Abbot, Andrew Heath, Christopher Kopp and Elizabeth Premo for help
310 in maintaining the field experiment along with Melanie Merkley and Rasha Al Sawad for sample
311 collection and laboratory assistance. This work was performed at the San Diego State
312 University's Santa Margarita Ecological Reserve and we thank Pablo Bryant for site access and
313 maintenance. This material is based upon work supported by the National Science Foundation
314 Division of Environmental Biology grant (DEB 1154082).

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Table 1. Soil physicochemical properties (mean) in native- and exotic-dominated plots by rainfall treatment prior the beginning of the experiment.

Plant community type	Native			Exotic		
Rainfall treatment	50%	100%	150%	50%	100%	150%
Sand (g g^{-1})	0.3	0.3	0.3	0.2	0.3	0.3
Silt (g g^{-1})	0.5	0.5	0.6	0.6	0.5	0.6
Clay (g g^{-1})	0.2	0.2	0.2	0.2	0.2	0.2
K (mg g^{-1})	0.2	0.1	0.2	0.2	0.2	0.2
Ca (mg g^{-1})	3.1	2.6	2.9	3.0	2.8	3.1
Mg (mg g^{-1})	1.3	1.3	1.3	1.3	1.3	1.5
Al (mg g^{-1})	2.7	2.2	2.7	2.7	2.7	2.6
pH (saturation paste)	6.1	6.2	6.1	6.2	6.1	6.2
Soil organic matter (g g^{-1})	0.1	0.1	0.1	0.1	0.1	0.1

Table 2. Summary of p-values for the linear mixed model for effects of rainfall treatments, plant community type, season, year and the interaction on soil N parameters (there were no significant 4-way interaction).

Factor(s)	NH4	NO3	Net Min	Net Nit	Leaching
Rainfall treatment	0.0013*	0.0059*	0.2667	0.3516	0.2499
Plant community type	0.0168*	<0.0001*	0.1316	0.2969	<0.0001*
Season	0.0006*	0.0010*	N.A	N.A	N.A
Year	0.0002*	<0.0001*	0.0265*	0.1548	<0.0001*
Rain:Plant	0.1983	0.2178	0.0698.	0.3176	0.4442
Rain:Season	0.2038	0.1680	N.A.	N.A.	N.A.
Plant:Season	0.5998	0.7013	N.A.	N.A.	N.A.
Rain:Year	0.1617	0.2553	0.7984	0.7497	0.3474
Plant:Year	0.1145	<0.0001*	0.2107	0.1816	<0.0001*
Season:Year	0.0863.	0.0029*	N.A.	N.A.	N.A.
Rain:Plant:Season	0.0365*	0.1335	N.A.	N.A.	N.A.
Rain:Plant:Year	0.7893	0.6098	0.7927	0.8729	0.5234
Rain:Season:Year	0.5423	0.5563	N.A.	N.A.	N.A.
Plant:Season:Year	0.7303	0.0523.	N.A.	N.A.	N.A.

513

514 **Figure Legends**

515 **Figure 1.** Inter-annual variation of soil: (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
516 nitrification for each plant community type from 2013 to 2016 across rainfall treatments (no
517 plant community type \times rainfall treatment interaction). Yellow bars = exotic plots; green bars =
518 native plots. Error bars represent the standard error of the mean.

519 **Figure 2.** Intra-annual variation of (a) NH_4^+ and (b) NO_3^- for each plant community type from
520 winter (Wi) to summer (Su). Yellow bars = exotic plots; green bars = native plots. Error bars
521 represent the standard error of the mean.

522 **Figure 3.** Drought severity effects on (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d)
523 nitrification for each plant community type. Yellow bars = exotic plots; green bars = native plots.
524 Error bars represent the standard error of the mean.

525 **Figure 4.** Inter-annual variation of leaching (DIN in resin bags) and deep soil NO_3^-
526 concentrations during the 2015 summer season. Error bars represent the standard error of the
527 mean.

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Figure 1

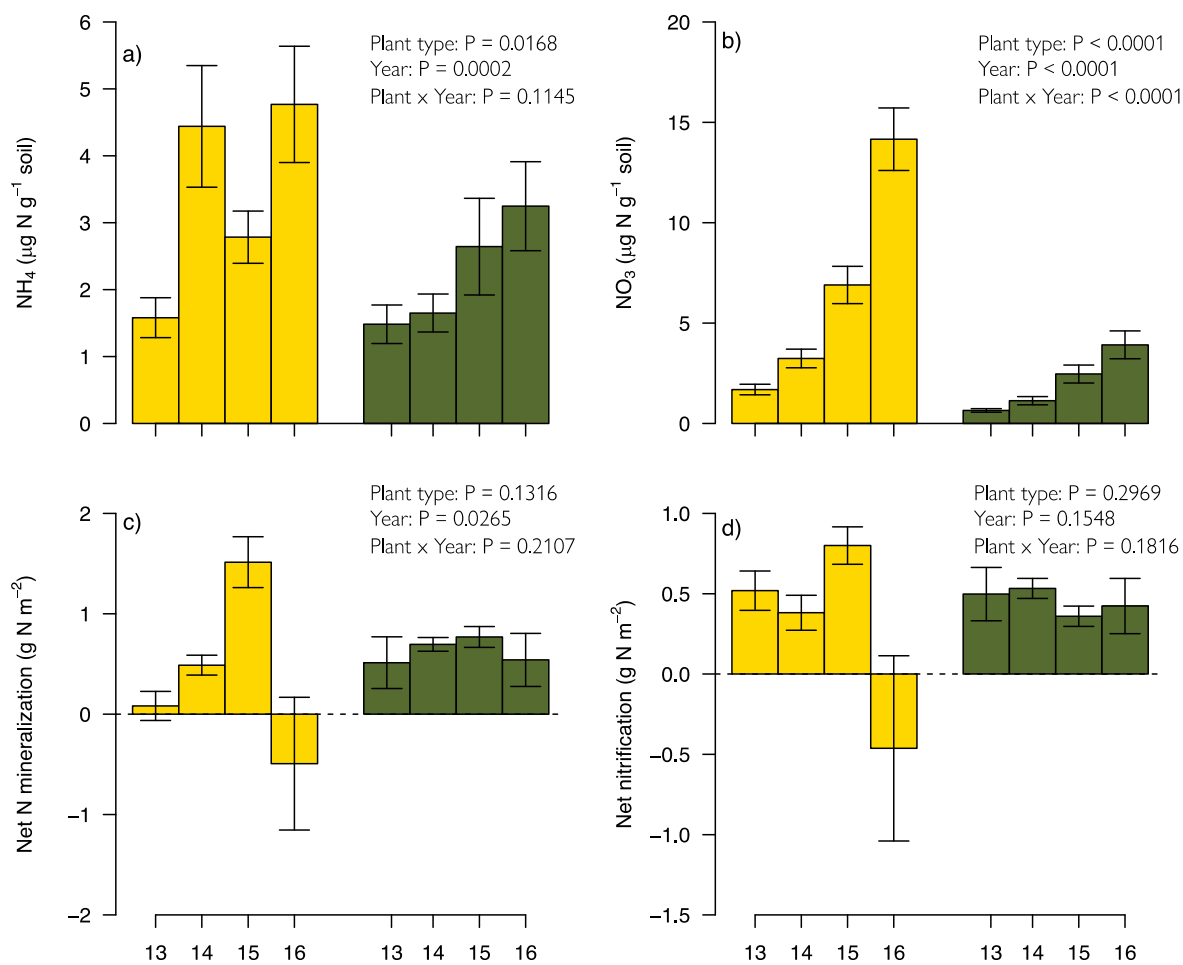
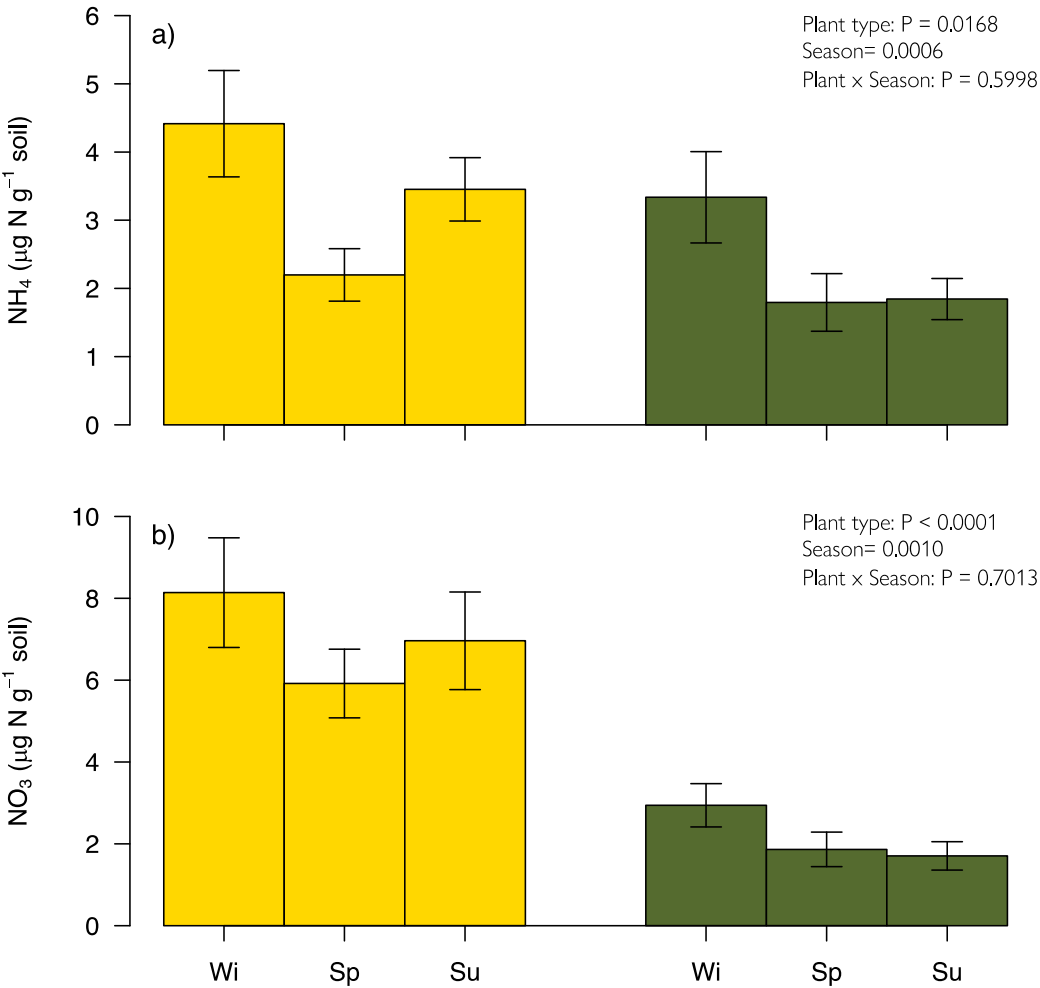
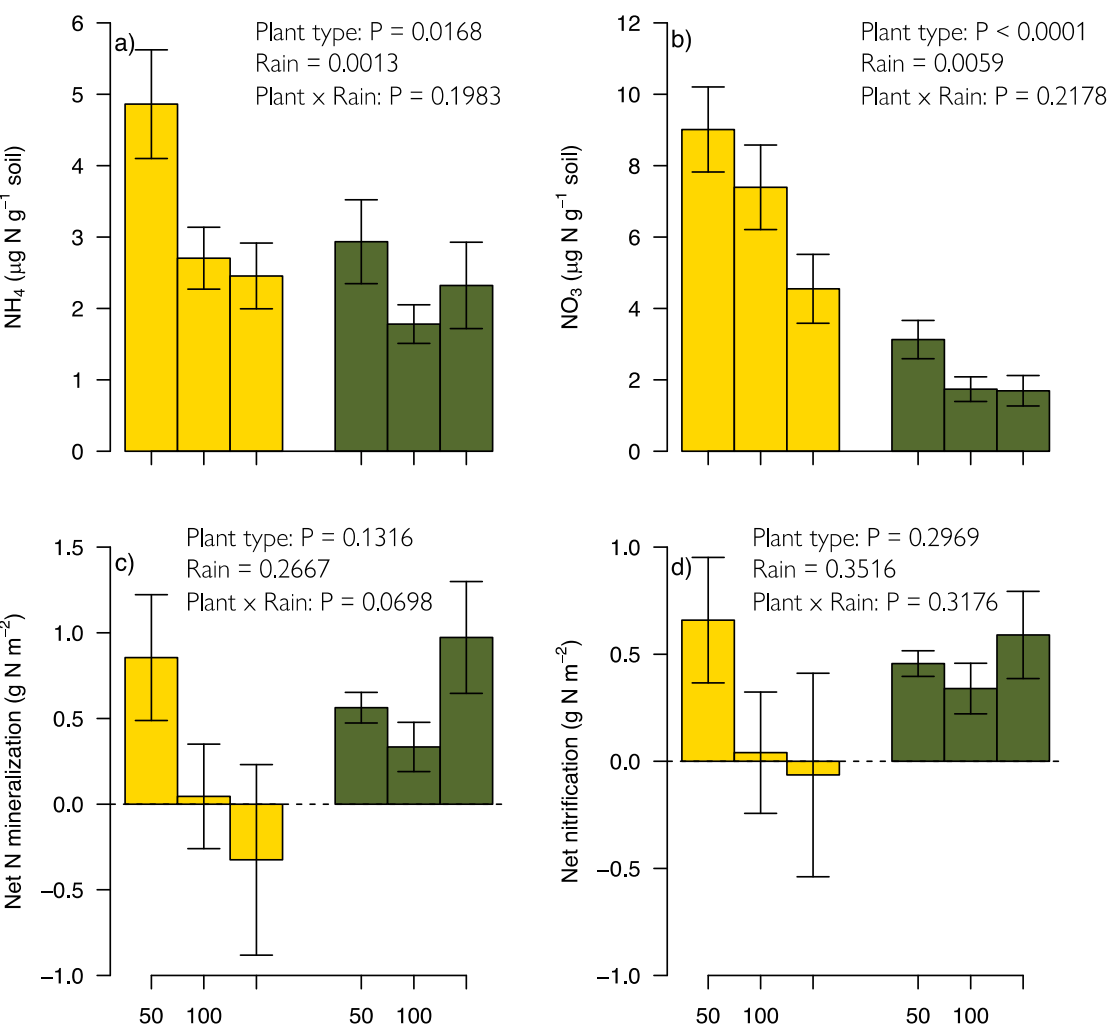


Figure 2



549

550 **Figure 3**



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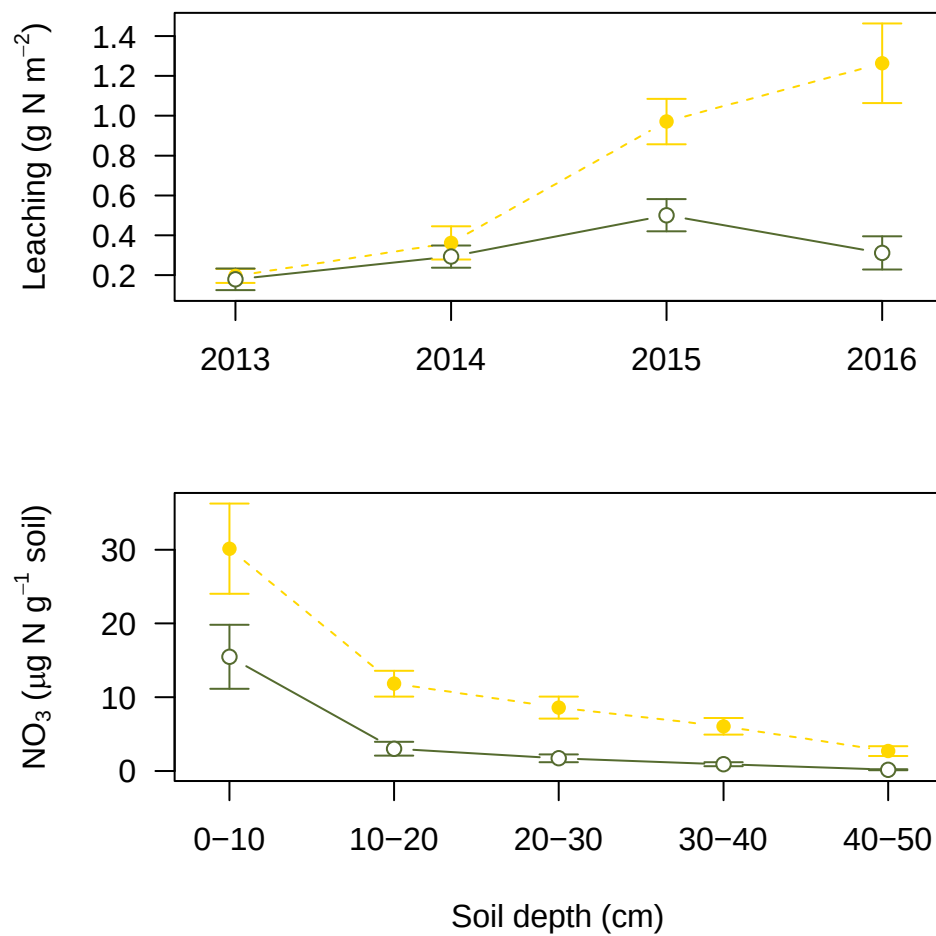
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556 **Figure 4**



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Highlights

- Reduced leaching and microbial N cycling promote soil N accumulation during droughts
- Droughts and exotic plants result in the enhanced accumulation of soil N
- Exotic plants facilitate increases in N leaching upon soil rewetting

Title: Exotic herbaceous species interact with severe drought to alter soil N cycling in a semi-arid shrubland

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Keywords: Climate change, Exotic species invasion, Coastal sage scrub; Soil nitrogen

Abstract

Mediterranean-type ecosystems are increasingly threatened by climate change and exotic annual species, jeopardizing the native communities and their global biodiversity. In these systems, soil nitrogen (N) limits net primary production, and its availability can be influenced by both of these stressors. To understand the interactive effects of droughts and exotic herbaceous species on soil N, we monitored the temporal variability of soil inorganic N, net N mineralization, net nitrification, and NO_3^- leaching under native- and exotic-dominated stands exposed to rainfall manipulation plots in a Mediterranean-type shrub-dominated community. Increasing drought severity resulted in the accumulation of soil NH_4^+ and NO_3^- , with a more pronounced increase in exotic-dominated plots. Increased net N mineralization and net nitrification and reduced leaching losses were observed as mechanisms of inorganic N accumulation. In comparison to soils under native plants, soils under exotic plants had enhanced leaching losses upon soil rewetting. We propose that distinct traits of exotic annual herbaceous species associated with higher N inputs, faster turnover, and reduced temporal uptake determine the changes in N cycling in response to droughts. Severe droughts and exotic plants may produce a larger, more vulnerable pool of N that is prone to losses while providing a competitive advantage to promote exotic growth in these N-limited ecosystems.

Introduction

Nitrogen (N) cycling is being altered globally more than any other essential nutrient (Fields, 2004). Agriculture and fossil fuel combustion are increasing reactive N in the

atmosphere (Galloway et al., 2008), droughts are increasing the ecosystems' N losses (Homyak et al., 2017), and invasive plants are altering ecosystems' N pools and fluxes (Ehrenfeld, 2003; Liao et al., 2008). These N alterations represent a major challenge particularly in ecosystems where N limits net primary production (Delgado-Baquerizo et al., 2014), and its availability is being threatened by multiple co-occurring global change drivers (Chapin et al., 2000; Sala et al., 2000). For example, semi-arid shrublands are currently experiencing invasions by exotic herbaceous species (Minnich and Dezzani, 1998; Yu et al., 2016), more intense and prolonged droughts (Spinoni et al., 2014; Swain et al., 2018), and elevated N deposition (Valliere et al., 2017), constituting multiple stressors that may have large impacts on N cycling.

In semi-arid ecosystems, N's limited accessibility is controlled by the temporal dynamics of water availability (Reichmann et al., 2013; Shen et al., 2008). During wet periods, N becomes accessible for microbial and plant assimilation and transformations, which contrasts to natural periods of drought when decreases in diffusion and biological uptake result in the accumulation of immobile N replenished from dead biomass and dry deposition (Cregger et al., 2014). The synchronized immobilization by microbes and plants during wet periods coupled with limited diffusivity during dry periods promotes a closed N cycle (recycling) in these systems (Homyak et al., 2016; Joergensen and Wichern, 2018). However, an asynchrony/decoupling between soil N availability and plant and microbial processes creates N leaks, particularly when dry soils rewet outside of the plant growing season (Homyak et al., 2016). Drought-tolerant microbial transformations and a lack of plant demand during ephemeral periods with mobile N availability favors N losses via leaching and gaseous pathways (Dijkstra et al., 2012). However, losses due to

64 this open N cycling have been reported to be low or limited to intermittent rain pulses in semi-
65 arid ecosystems (Evans and Burke, 2012; Jongen et al., 2013).

66
67 More intense and prolonged droughts due to climate change are expected to promote
68 open N cycling in semi-arid shrublands via sustained N processing during periods of low plant
69 uptake (Evans and Burke, 2012; Homyak et al., 2016). Invasions by exotic herbaceous species
70 have also been reported to increase soil N availability and cycling rates (Blank, 2008; Hawkes et
71 al., 2005; Wolkovich et al., 2010) by possessing traits associated with faster N turnover (Castro-
72 Díez et al., 2014). In semi-arid ecosystems this excess of N availability has been associated with
73 detrimental environmental effects, such as increasing emissions of nitrogenous greenhouse gases
74 (Homyak et al., 2016), eutrophication, fire frequency (Fenn et al., 2003), and competitive
75 advantages for exotic plant species (Laungani and Knops, 2009; Liu et al., 2018). While soil N
76 cycling alterations in response to droughts and plant invasions in semi-arid shrublands have been
77 evaluated, a mechanistic understanding of the interactive effects of these co-occurring global
78 change drivers is not only limiting our ability to model ecosystem responses to multiple global
79 change drivers but also limits improvements to conservation efforts aimed at maintaining
80 ecosystem services (Alba et al., 2017; Laungani and Knops, 2009; Valliere, 2016). Levels of
81 uncertainty arise from challenges in the evaluation of temporally dynamic abiotic and biotic
82 drivers (Alba et al., 2017) and the intrinsic variability of dryland ecosystems that are
83 characterized by distinct plant community types (Fenn et al., 2003) and hydrologic regimes
84 (D'Odorico and Bhattachan, 2012).

Here we present the results of a four-year rainfall manipulation field study in a coastal sage scrub community (CSS), an understudied semi-arid Mediterranean-type, shrub-dominated ecosystem distributed from coastal central California, USA to northern Baja California, Mexico, that has been invaded by exotic herbaceous species (Minnich and Dezzani, 1998) and experienced multi-year extreme drought conditions (Swain et al., 2018). Established exotic annual grasses and forbs species differ from dominant CSS native perennial semi-deciduous shrubs not only in growth form and phenology but also in distinct drought strategies (Balachowski et al., 2016; Puritty et al., 2019; Wainwright et al., 2012; Wolkovich et al., 2010). While CSS native shrubs are adapted to persist through seasonal droughts by partial senescence of mature foliage that is released late in the dry season, exotic annual species exhibit faster growth and earlier complete senescence before experiencing dehydration (Balachowski et al., 2016). The monitoring of temporal variability of soil inorganic N, net N mineralization, net nitrification, and NO_3^- leaching under native- and exotic-dominated rainfall manipulated plots allowed us to assess interactive mechanisms of soil N alterations. Combining field manipulations and functional trait approaches has become a powerful tool to improve our mechanistic understanding of ecosystem responses and feedbacks to multiple global change drivers (Boyd et al., 2018; Laungani and Knops, 2009). We hypothesized that the earlier senescence of exotic species, and their more easily decomposable litter compared to native species, would enhance soil N availability and result in more open cycling in response to droughts (Liao et al., 2008).

Materials and Methods

This study was conducted from 2013 – 2016 at the Santa Margarita Ecological Reserve (SMER) rainfall manipulation experiment (33.44128 N, 117.1644 W), located at the Riverside-San Diego county line, California, USA. The region has a semi-arid Mediterranean-type climate with cool wet winters and hot dry summers with a compared 30-year average of 358 mm yr⁻¹ of rainfall. The site was a grazed pasture prior to the establishment of the Reserve in 1962. Currently, the area is a heterogeneous matrix of native mature shrubs such as *Artemisia californica* and *Salvia mellifera* interspersed with exotic annual species such as *Centaurea melitensis*, *Hirschfeldia incana*, *Erodium cicutarium* and *Bromus madritensis*. All plots were located on similar soil, classified as Las Posas loam, which are well drained with an argillic horizon under the top soil (<http://websoilsurvey.sc.egov.usda.gov>). Soil analyses at the beginning of the experiment showed no differences in physicochemical properties between plots (Table 1). We are therefore confident that the effects observed in this experiment did not arise from pre-experimental variability among plots. The field experiment consisted of 30 3 m x 3 m rainfall shelters covered with a clear, polycarbonate roof permitting light transmission. Half of the plots were dominated by the native mature shrubs, while the remaining plots were dominated by exotic annual species. To implement the rainfall manipulation, rainfall collected from the rain shelters was re-applied to plots using sprinkler heads at three rainfall levels (in each vegetation type, 5 replicates of 50%, 100%, and 150% of ambient rainfall) after rainfall events. During the sampling period, the region experienced below average annual rainfall with the ambient rainfall plots receiving 157, 125, 139, and 156 mm yr⁻¹ during water years 2013 – 2016, respectively. Due to the lack of rainfall, the 50% and 100% rainfall treatments represented severe drought and drought conditions, respectively, and the 150% treatment corresponded to an average water year.

Water year 2015 received major Pacific storms and summer rains associated with the 2015 – 2016 El Niño event.

N pools

To evaluate temporal variability in soil inorganic N, we sampled soils from all plots during the winter (rainy season: October-February), spring (peak plant biomass: March-April), and summer (dry season: May-September) over the 4-year sampling period, resulting in 12 sampling events. Soil was sampled to a depth of 15 cm using a 5 cm diameter x 15 cm deep slide hammer soil core sampler. Samples were stored in a cooler for transportation and processed within 24 h of collection. All visible stones, roots, and litter were removed with forceps prior to analysis. Dissolved NH_4^+ and NO_3^- were determined in 0.5M potassium sulfate extracts using the indophenol method and by vanadium (III) reduction, respectively, and measured on a spectrophotometer (Miranda et al., 2001).

Net N mineralization, net nitrification and leaching

Net N mineralization and nitrification were assessed using in situ soil core incubations with 4 cm diameter and 10 cm depth PVC tubes (DiStefano and Gholz, 1986). Two cores were inserted into the soil inside the plots of 3 out of the 5 replicates for each plant community type and each precipitation manipulation ($n_{natives}=9$ vs. $n_{exotics}=9$) at the beginning of the winter

seasons. One of the cores was immediately removed for analyses (T_0) and the other core, which was fitted with an ion exchange resin bag at the bottom of the tube, was removed during the summer season (T_F). Dissolved NH_4^+ and NO_3^- were analyzed in the potassium sulfate extracts from soils in the cores and in 2 M KCl extracts from the ion exchange beads in the resin bags. Annual N cycling measurements included: (1) N mineralization, calculated as the difference in DIN ($NH_4^+ + NO_3^-$) concentrations between T_F and T_0 plus the DIN in resin bags, (2) nitrification, calculated as the difference in NO_3^- concentrations between T_F and T_0 in resin bags, and (3) leaching as the amount of DIN in resin bags (T_F). Although resin bags may not retain all NH_4^+ and NO_3^- flowing down the core, the amount of DIN in resin bags provides a reliable estimate of N leaching (Bhogal et al., 1999). To compliment leaching measurements, immediately after the 2015 summer storms, we collected soil samples to a depth of 50 cm and measured dissolved NO_3^- on water extracts using ion-chromatography (Thermo ICS-5000+).

Statistical analyses

Data analysis was performed in R version 3.3.3 (R Core Team, 2017). To determine the effects of rainfall treatment, plant community type, season, and year and their interactions on soil NH_4^+ , NO_3^- , N mineralization, nitrification, and leaching, mixed-effects repeated measures analysis of variance (RM-ANOVA) was employed using the lme function within the ‘nlme’ package (Pinheiro et al., 2018). Linear model assumptions of normality and homoscedasticity were tested using Shapiro-Wilk and Levene’s test and observed visually using Q-Q plots and plotting residuals against fitted values, respectively. When significant effects were found, multiple comparisons between treatments, seasons, and years were performed using the glht

function within the ‘multcomp’ package (Hothorn et al., 2008). P-values less than 0.05 were considered significant.

Results

Nitrogen pools

We detected significant differences in soil NH_4^+ and NO_3^- concentrations between years ($p_{NH_4^+} = 0.0002$ and $p_{NO_3^-} < 0.0001$), seasons ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.01$), rainfall treatments ($p_{NH_4^+} = 0.001$ and $p_{NO_3^-} = 0.006$), and plant community types ($p_{NH_4^+} = 0.02$ and $p_{NO_3^-} < 0.0001$; Table 1). Mean annual soil NH_4^+ and NO_3^- concentrations increased over the 4-year sampling period under both plant community types, with a more pronounced increase in exotic-dominated plots (Fig. 1a-b). In 2016, annual average soil NH_4^+ was 3.1 and 2.5 times higher in exotic- and native-dominated plots, respectively, when compared to 2013 (Fig. 1a). Annual average soil NO_3^- was 6.0 and 8.4 times higher in native- and exotic-dominated plots, respectively, in 2016 when compared to 2013 (Fig. 1b). Increases in soil NH_4^+ and NO_3^- concentrations were also observed during the winter season (Fig. 2a-b) and in the 50% rainfall treatment under both plant communities (Fig. 3a-b). Soil NH_4^+ in the 50% treatment (severe drought) was 2.0 and 1.3 times higher in exotic- and native-dominated plots, respectively, when compared to the 150% treatment (Fig. 3a). Soil NO_3^- was 2.0 and 1.8 times higher in exotic- and native-dominated plots, respectively, in the 50% treatment when compared to the 150% treatment (Fig. 3b). Soil NH_4^+ concentrations were increased in exotic plots by approximately 43% based

on average values across the entire time of observations (estimated mean \pm SD, native-dominated: $2.4 \pm 3.6 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $3.4 \pm 4.2 \mu\text{g N g}^{-1}$ soil). Soil NO_3^- concentrations were increased in exotic plots by approximately 219% (estimated mean \pm SD, native-dominated: $2.2 \pm 3.2 \mu\text{g N g}^{-1}$ soil vs. exotic-dominated: $7.0 \pm 8.2 \mu\text{g N g}^{-1}$ soil).

Net N mineralization, net nitrification and leaching

Net N mineralization was influenced by year ($p = 0.03$), and there was a moderately significant interaction between rainfall treatment and plant community type ($p = 0.07$; Table 1). Annual net N mineralization was more variable in exotic-dominated plots (Fig. 1c). In exotic plots, net N mineralization rates increased from 2014 to 2015 and decreased from 2015 to 2016. Highest net N mineralization occurred in 2015 in exotic-dominated plots (Fig. 1c). During 2016, net N mineralization was negative (i.e. net immobilization) in exotic plots (Fig. 1c). Net N mineralization differed between the two community types in response to drought. In exotic-dominated plots net N mineralization increased with increasing drought intensity, while in native plots the opposite pattern was present with the highest levels observed in the 150% treatment (Fig. 3c).

While we did not detect significant effects of rainfall treatment, plant community type, or year on net nitrification (Table 2), similar patterns to net N mineralization rates were observed (Figure 1d & 3d). Highest net nitrification rates occurred in 2015 in exotic-dominated plots, and

negative net nitrification was observed in 2016 (Fig. 1d). The response of net nitrification to drought differed in direction between the two community types, although this was non-significant. In exotic-dominated plots net nitrification increased with drought intensity, and in native plots highest levels of net nitrification were found in the 150% treatment (Fig. 3d).

Leaching (DIN in resin bags) was significantly influenced by the interaction between plant community type and year ($p < 0.0001$). In soils under native plants, leaching was higher during 2015 when compared with 2013, 2014, and 2016 (Fig. 4a). In soils under exotic plants, leaching was higher during 2015 and 2016 when compared with 2013 and 2014 (Fig. 4a). During 2015 and 2016 leaching was 1.9 and 4.1 times higher in exotic-dominated plots when compared to native plots, respectively. After the 2015 summer rains we measured a 1.9, 3.9, 5.0, 6.7, and 16.4 times increase in soil dissolved NO_3^- concentrations in exotic plots when compared to native plots at 0-10, 10-20, 20-30, 30-40, and 40-50 cm soil depths, respectively (Fig. 4b).

Discussion

Soil inorganic N accumulation under drier conditions

Consistent with the generalization that drier conditions facilitate inorganic N accumulation (Evans and Burke, 2012; Homyak et al., 2017, 2016), in this study we found higher levels of soil NH_4^+ and NO_3^- concentrations in response to increasing drought severity due both to rainfall manipulations and over the 4-year period of below average annual rainfall under both

plant community types. We observed several patterns that provide insight into the mechanisms of soil inorganic N accumulation. First, annual rates of net N mineralization and nitrification rates were observed to be resistant to drought severity on average (rainfall treatments, Table 2), and net nitrification did not differ significantly during the 4 consecutive years of below average annual rainfall, suggesting statistically unaltered contributions to the supply of NH_4^+ and NO_3^- concentrations during dry periods. The resistance of microbial processes to low water potentials has been reported particularly in dryland ecosystems due to their natural exposure to seasonal droughts (Canarini et al., 2017; Drigo et al., 2017). However, it is also possible that there were seasonal changes in N cycling rates that were not detected with our annual measures. Increased inorganic N in response to drought may have also been caused by other drought-tolerant microbial N sources, such as asymbiotic N fixation, (Boring et al., 1988), or N deposition, particularly dry deposition (Valliere et al., 2017). In the location of our study, N deposition occurs at levels ranging from 9-11 kg N $ha^{-1}yr^{-1}$ (Fenn et al., 2010). Secondly, we observed a significant increase in leaching during 2015 (wettest year) when compared to previous years under both plant community types. This pattern suggests inorganic N accumulates through reduced leaching losses during drier conditions. Nitrate is highly mobile, and its diffusivity has been previously reported limited under drought conditions (Evans and Burke, 2012). Lastly, differences in soil inorganic N concentrations and leaching between exotic- and native-dominated plots suggest that plant-derived mechanisms influence N accumulation.

Soil inorganic N accumulation as affected by invasion

In comparison to soils under native plants, soils under exotic herbaceous species were observed to have higher soil inorganic N accumulation. We propose that exotic herbaceous species increased soil inorganic N availability by making N cycling more rapid. Litter N release may have been faster from the exotic herbaceous species because of their higher litter quality and their earlier and complete senescence of biomass. This explanation is consistent with the generalization that invasive plants increase N pools by possessing traits associated with faster N turnover (Castro-Díez et al., 2014; Liao et al., 2008). Additionally, invasion by exotic species may accelerate N release to soils via increased litter susceptibility to UV photodegradation compared with native litter (Esch et al., 2019a). Vegetation structure could also play a role, aboveground cover by exotic herbaceous species when compared with native shrubs could enhance the effects of solar radiation on litter decomposition. Plant traits associated with N-fixation have been proposed as another mechanism by which invasive plants enhance N pools (Castro-Díez et al., 2014). While not evaluated in this study, higher asymbiotic N fixation activity may be a mechanism by which the non N-fixing exotic species could have increased soil N inputs (Castro-Díez et al., 2014; Eisele et al., 1989; Mack et al., 2001). An increase in the relative abundance of Cyanobacterial DNA sequences has been found in our exotic-dominated plots (Pérez Castro et al., 2019), which are associated with N fixation (Green et al., 2008).

The interaction of invasion and dry conditions

Contrary to soils under native-dominated plots, we observed a moderate positive relationship between drought severity and N accumulation and net transformations under exotic-dominated plant communities. We proposed that accelerated N cycling by exotic herbaceous

species coupled with drought-induced increases in N accumulation generated a larger inorganic N pool. However, significant decreases in soil NH_4^+ from 2014 to 2015 (the relatively wet year), and increases in N leaching and higher deep soil NO_3^- levels during 2015 in soils under exotic species when compared to soils under native species, suggest that soils under exotic plants did not retain the accumulated N. We speculate that the shorter phenology of the exotic annual species (Esch et al., 2019b) and reduced N uptake (demand) during water inputs increased its vulnerability to leaching losses. This is consistent with the hypothesis that drought-induced soil inorganic N accumulation is more vulnerable to losses upon rewetting (Evans and Burke, 2012).

Conclusions

In this experimental semi-arid shrubland, we found that plant functional traits are key factors in providing mechanistic explanations of soil responses and feedbacks to environmental disturbances. In comparison to soils under native woody plants, we observed that soils under exotic herbaceous plants had enhanced inorganic N accumulation and leaching losses after experiencing severe drought conditions. Our findings indicate that distinct morphological, physiological, and phenological traits of annual herbaceous species, associated with N higher inputs, faster turnover, and reduced temporal uptake, play a major role in N pools and fluxes. Nitrogen alterations under severe droughts and exotic plants likely produce a larger pool of N that is more vulnerable to increased leaching and may provide a competitive advantage to promote exotic growth and invasiveness success in these N-limited ecosystems (Liu et al., 2018; Vourlitis, 2017). While this study suggests N transformation rates as mechanisms of inorganic N accumulation, future efforts that measure gross N production are needed to confirm this speculation and better understand microbially mediated N-cycling processes.

307

308 **Acknowledgments**

309 We thank Rachel Abbot, Andrew Heath, Christopher Kopp and Elizabeth Premo for help
310 in maintaining the field experiment along with Melanie Merkley and Rasha Al Sawad for sample
311 collection and laboratory assistance. This work was performed at the San Diego State
312 University's Santa Margarita Ecological Reserve and we thank Pablo Bryant for site access and
313 maintenance. This material is based upon work supported by the National Science Foundation
314 Division of Environmental Biology grant (DEB 1154082).

315

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Table 1. Soil physicochemical properties (mean) in native- and exotic-dominated plots by rainfall treatment prior the beginning of the experiment.

Plant community type	Native			Exotic		
Rainfall treatment	50%	100%	150%	50%	100%	150%
Sand (g g^{-1})	0.3	0.3	0.3	0.2	0.3	0.3
Silt (g g^{-1})	0.5	0.5	0.6	0.6	0.5	0.6
Clay (g g^{-1})	0.2	0.2	0.2	0.2	0.2	0.2
K (mg g^{-1})	0.2	0.1	0.2	0.2	0.2	0.2
Ca (mg g^{-1})	3.1	2.6	2.9	3.0	2.8	3.1
Mg (mg g^{-1})	1.3	1.3	1.3	1.3	1.3	1.5
Al (mg g^{-1})	2.7	2.2	2.7	2.7	2.7	2.6
pH (saturation paste)	6.1	6.2	6.1	6.2	6.1	6.2
Soil organic matter (g g^{-1})	0.1	0.1	0.1	0.1	0.1	0.1

Table 2. Summary of p-values for the linear mixed model for effects of rainfall treatments, plant community type, season, year and the interaction on soil N parameters (there were no significant 4-way interaction).

Factor(s)	NH4	NO3	Net Min	Net Nit	Leaching
Rainfall treatment	0.0013*	0.0059*	0.2667	0.3516	0.2499
Plant community type	0.0168*	<0.0001*	0.1316	0.2969	<0.0001*
Season	0.0006*	0.0010*	N.A	N.A	N.A
Year	0.0002*	<0.0001*	0.0265*	0.1548	<0.0001*
Rain:Plant	0.1983	0.2178	0.0698.	0.3176	0.4442
Rain:Season	0.2038	0.1680	N.A.	N.A.	N.A.
Plant:Season	0.5998	0.7013	N.A.	N.A.	N.A.
Rain:Year	0.1617	0.2553	0.7984	0.7497	0.3474
Plant:Year	0.1145	<0.0001*	0.2107	0.1816	<0.0001*
Season:Year	0.0863.	0.0029*	N.A.	N.A.	N.A.
Rain:Plant:Season	0.0365*	0.1335	N.A.	N.A.	N.A.
Rain:Plant:Year	0.7893	0.6098	0.7927	0.8729	0.5234
Rain:Season:Year	0.5423	0.5563	N.A.	N.A.	N.A.
Plant:Season:Year	0.7303	0.0523.	N.A.	N.A.	N.A.

Figure Legends

Figure 1. Inter-annual variation of soil: (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d) nitrification for each plant community type from 2013 to 2016 across rainfall treatments (no plant community type \times rainfall treatment interaction). Yellow bars = exotic plots; green bars = native plots. Error bars represent the standard error of the mean.

Figure 2. Intra-annual variation of (a) NH_4^+ and (b) NO_3^- for each plant community type from winter (Wi) to summer (Su). Yellow bars = exotic plots; green bars = native plots. Error bars represent the standard error of the mean.

Figure 3. Drought severity effects on (a) NH_4^+ , (b) NO_3^- , (c) N mineralization, and (d) nitrification for each plant community type. Yellow bars = exotic plots; green bars = native plots. Error bars represent the standard error of the mean.

Figure 4. Inter-annual variation of leaching (DIN in resin bags) and deep soil NO_3^- concentrations during the 2015 summer season. Error bars represent the standard error of the mean.

Figure 1

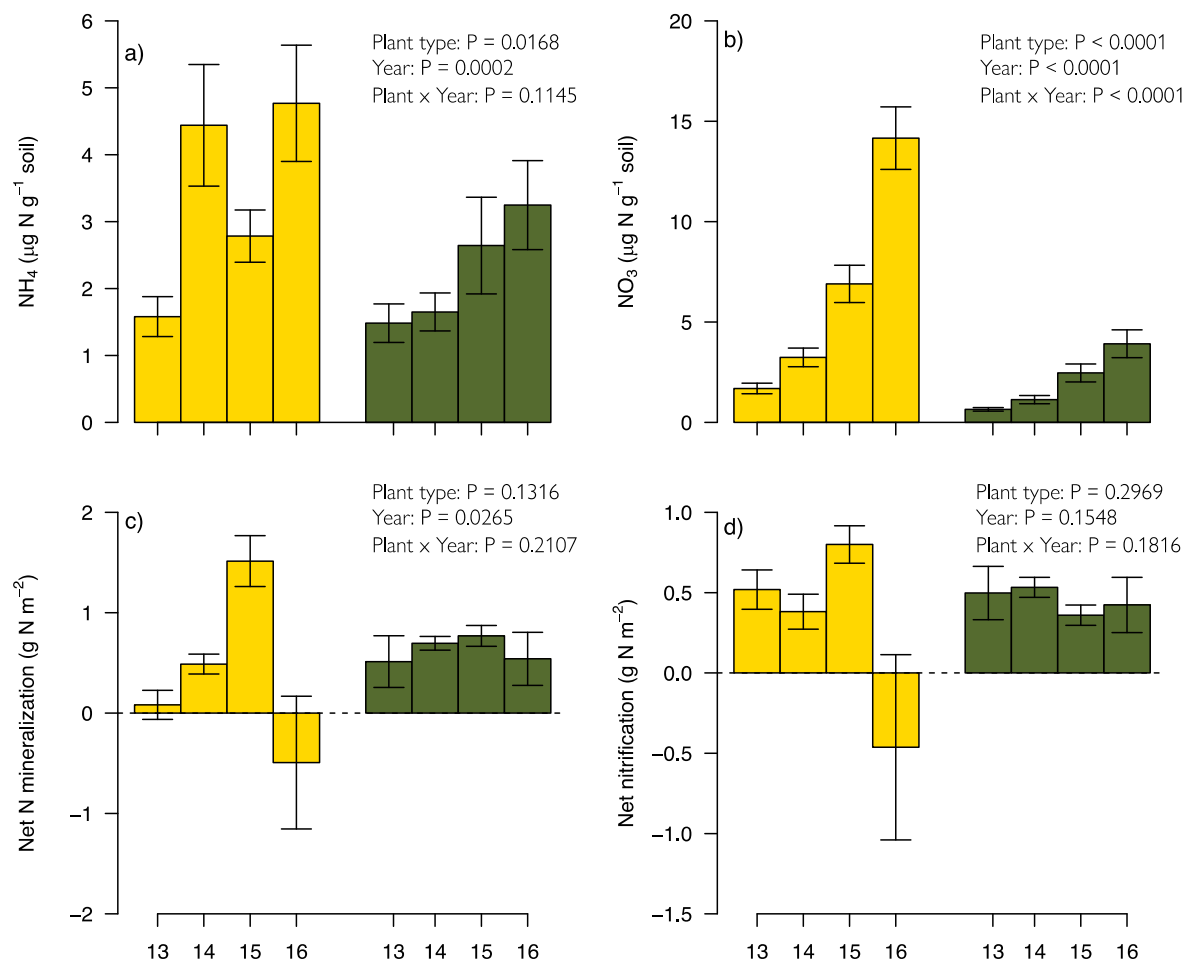
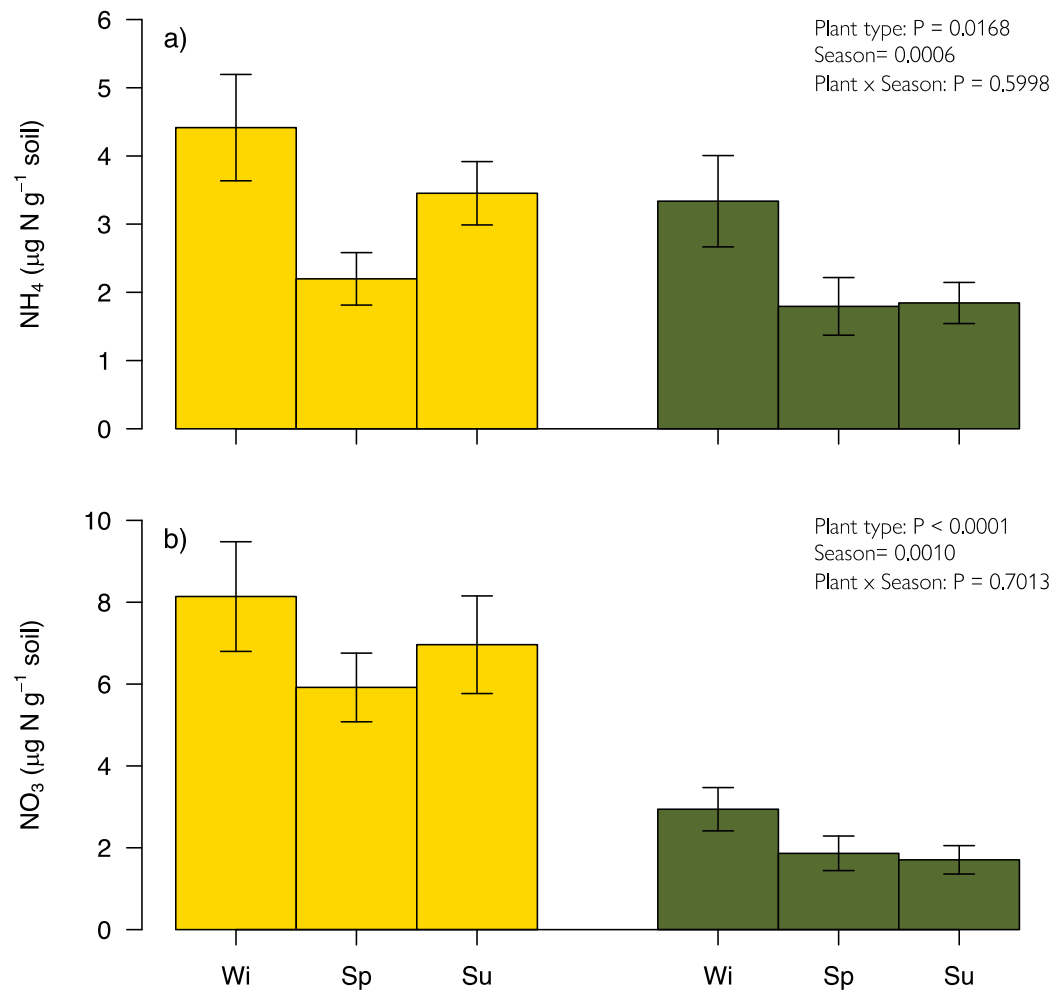


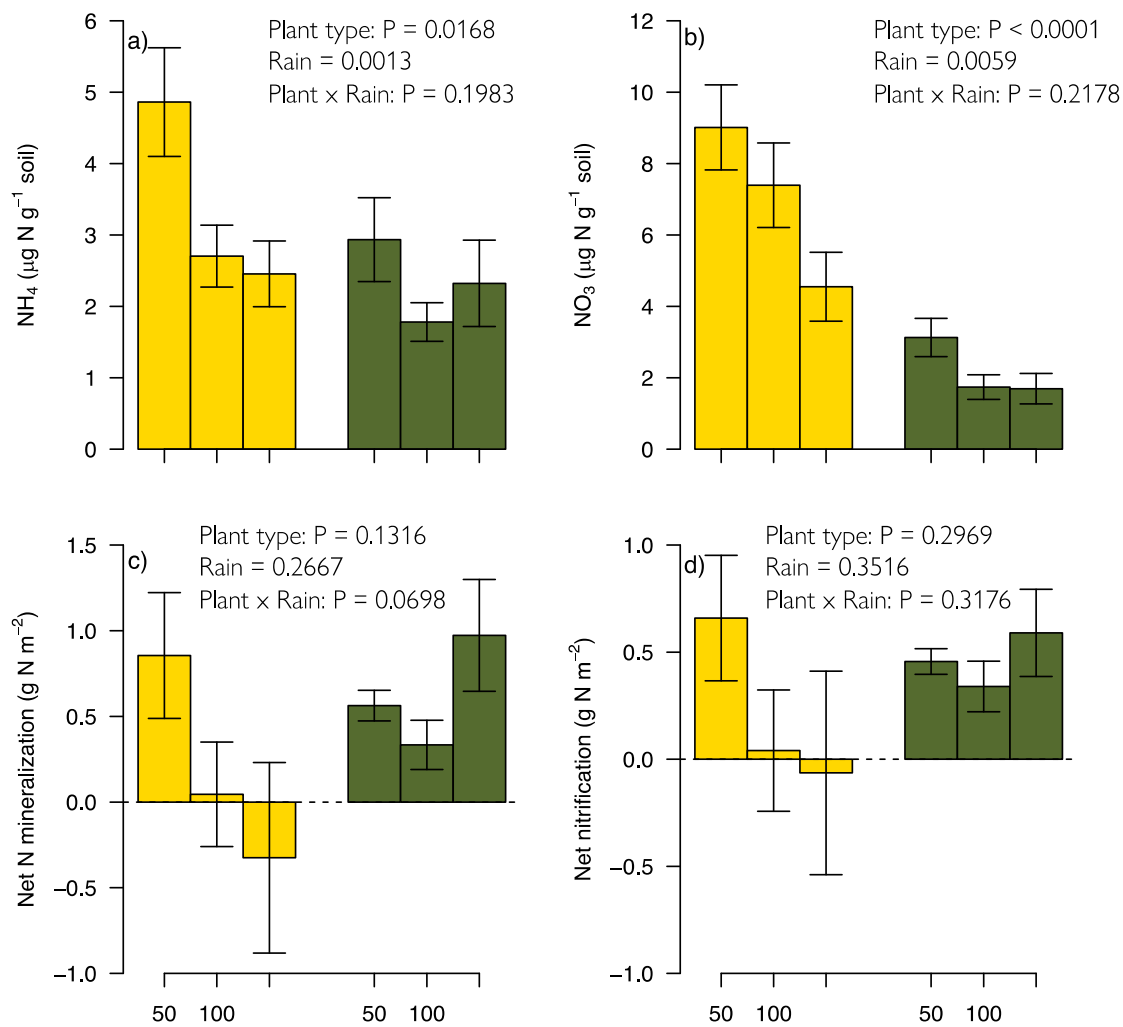
Figure 2



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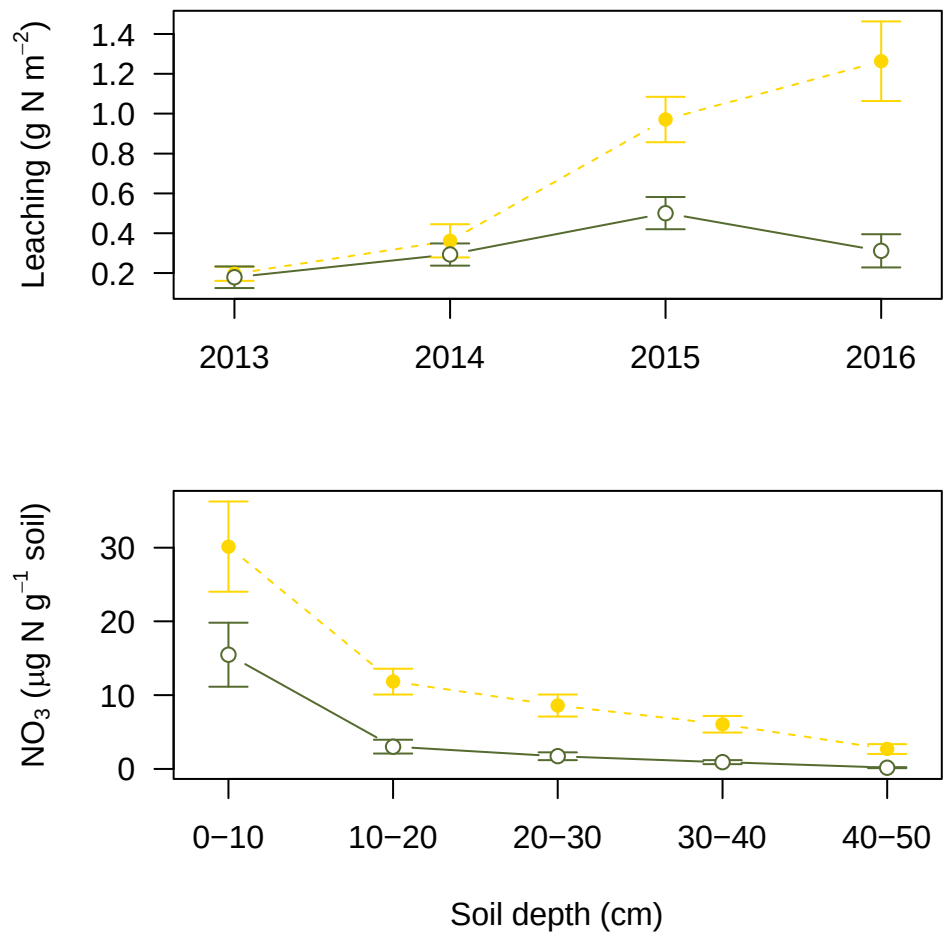
551 **Figure 3**



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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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